

The University of Chicago

COMPARATIVE STUDY OF
THE DEVELOPING AND ABORTING
FRUITS OF PRUNUS PERSICA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1933

By
THOMAS JEWELL HARROLD

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COMPARATIVE STUDY OF THE DEVELOPING AND ABORTING FRUITS OF PRUNUS PERSICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 459

THOMAS J. HARROLD

(WITH TWENTY-SEVEN FIGURES)

Introduction

A large percentage of the fruits of the peach fall from the trees before maturity, and this failure in development is a major problem among the growers of pome and stone fruits. The problem has been studied in considerable detail with the plum, the sour cherry, and the sweet cherry by DORSEY (7), BRADBURY (5), and TUKEY (12). With the exception of the work of DETJEN (6) and CONNORS (4), the peach has apparently been overlooked in this phase of the sterility problem.

The investigation covered the following points: (1) the situation in the peach as compared with the facts of abortion already known for the plum and cherry; (2) the careful comparison of developing and aborting fruits to aid in the discovery of the fundamental causes of this type of abortion; (3) the possible relation of the three growth periods of the peach, also recognized by other workers, to abortion and subsequent dropping of fruits.

Materials and methods

The Carman variety of peach (*Prunus persica* Sieb. & Zucc.) was used in this study. Material was collected in the College orchard at Athens, Georgia, during the season of 1933. The first collections were made about one week before full bloom, thereafter daily for a 30-day period, and then at intervals of two or three days until about three weeks before maturity. This material was of two sorts, that which was expected to develop and that which would probably not do so. Material of the developing series was secured by removing certain branches, which were taken to the laboratory and placed in water,

and then the pistils were dropped into fixing solution. Aborting material was collected on the tree as soon as it could be recognized, and by catching falling fruits in a net after jarring the tree with a padded pole. The material, both developing and aborting, was prepared by cutting away portions of the pericarp; in later stages by removing the seeds in order that fixation might be facilitated. A modification of Nawaschin's solution (BEAL 2) and formalin-acetic-alcohol were used.

Most of the sections were cut at $10\ \mu$, and stained in iron-alum haematoxylin or Flemming's triple stain. Measurements were made from fresh and from fixed material. The figures of the diagram in figure 1 represent in each case the average of five to twenty measurements.

Determination of total abortion was made by subtracting from the number of blossoms on certain limbs the number of fruits remaining on the same limbs after the third drop.

Microscopic study

The Carman variety of peach used in this experiment dropped approximately 70 per cent of the flowers and fruits during the season of 1933 (table I). The fall of flowers and fruits occurs in waves which are designated as the first, second, and third drops. Each wave of dropping may be differentiated according to time of occurrence and by morphological characters.

Assuming that the figures of table I may be applied to the entire orchard, it is evident that pollination is not a problem.

Each wave of dropping consists of flowers or fruits which proceed normally in development until a short time before falling. This is clearly explained by BRADBURY (5), who divides the developing and aborting fruits into three groups: "Group I . . . includes two lots: (a) those aborting fruits which will fall in the first drop and (b) the corresponding developing fruits, that is, fruits which are developing while the former are aborting. Group II is composed of fruits which were classed as developing fruits in Group I, but have become divisible into two lots: (a) aborting fruits which will fall in the second drop and (b) developing fruits. Similarly, Group III is made up of

fruits classed as developing fruits in Group II, but have become separable into two lots: (a) aborting fruits which will fall in the third drop and (b) developing fruits."

It was possible in this study to recognize aborting fruits several days before their fall.

FIRST DROP

The first wave of peach drops occurs during approximately a 3-week period beginning one to three days before full bloom. This drop consists of buds and of flowers with and without petals, con-

TABLE I
CARMAN VARIETY; 1933

	NUMBER OF BRANCHES	NUMBER OF FLOWERS AT FULL BLOOM	NUMBER OF FRUIT SET 2 WEEKS AFTER THIRD DROP	PERCENTAGE OF FLOWERS SETTING FRUIT
Open pollinated.	8	1153	378	31.9
Flowers brushed with pollen, not bagged.	6	991	301	30.3

taining shriveled pistils in some cases and plump pistils in others. Pericarp enlargement has not proceeded far enough to cause the shedding of the calyx cup. Fruits which develop lose the calyx cup about the middle of the first drop period (figs. 1, 2). Styles are present in both the dropped and the developing fruits. Some have two shriveled ovules as compared with the one plump ovule in all the developing fruits.

SECOND DROP

This wave of dropping occurs during approximately a 7-day period about the fifth week after bloom. It takes place during the middle of the first period of rapid development of the pericarp and integuments (fig. 1). Dropped fruits measure from 8 to 14 mm. in length, while the developing fruits measure 20 to 27 mm. (fig. 3). Color differences are apparent, the dropped fruits differing from developing fruits in being yellowish green as compared with the darker green of the latter. Both types are devoid of calyx cups.

THIRD DROP

This wave occurs during approximately a 7-day period about the seventh week after bloom. These fruits vary widely in size, measuring 25 to 30 mm. compared with an average length of 37 mm. in the developing fruits (figs. 1, 4). The flaccid and yellow-green appearance which characterized the second drop is also evident. The integuments have attained nearly the size found in mature fruits.

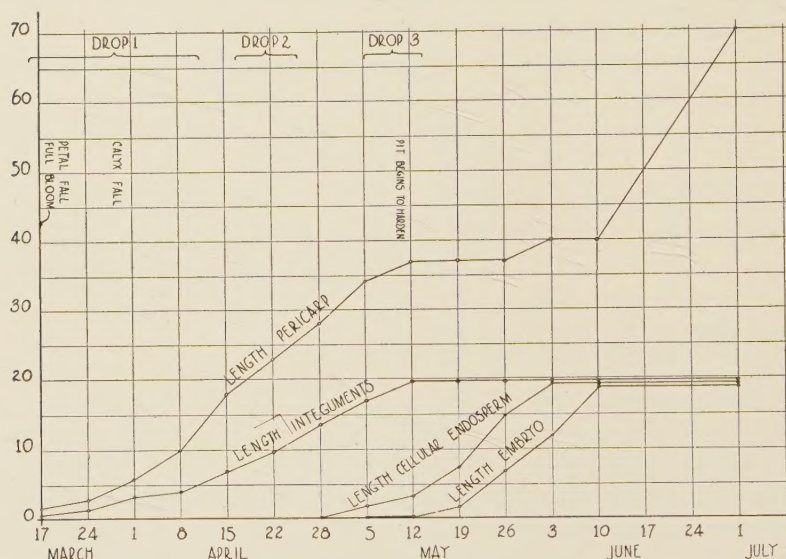


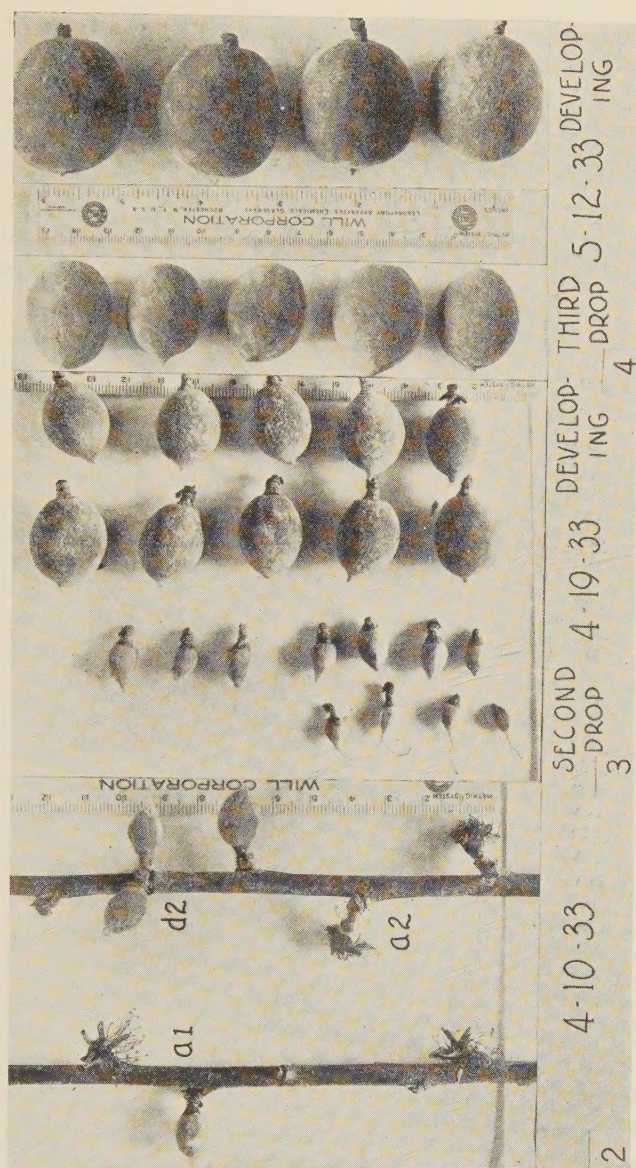
FIG. 1.—Diagram showing development of pericarp, integuments, cellular endosperm, and embryo in relation to time of drop.

Microscopic study of normal development

Correlation of parts of developing fruits with time of development is given in figure 1. This includes measurements of pericarp, integuments, cellular endosperm, and embryo. The diagram also shows the relation of each of the three drops to the developing fruits.

PERICARP

Enlargement of the pericarp of the peach does not proceed uniformly, but is divided into three stages (fig. 1). This has previously been recognized in the peach by CONNORS (3), DORSEY and Mc-



FIGS. 2-4.—Fig. 2, branch about the end of the first drop: *a1*, aborting first drop; *a2*, aborting second drop; *d2*, developing fruit. Fig. 3, condition during middle of second drop, showing aborting and developing fruits. Fig. 4, condition at about the end of the third drop, showing aborting and developing fruits.

MUNN (8), and LILLELAND (10); and in the sweet cherry by TUKEY (12). During the first period of eight weeks, the pericarp increased in length from 1.7 to about 37 mm., followed by a second period of four weeks in which the rate of development of the pericarp was retarded. The third period of three weeks is characterized by a final rapid enlargement of the pericarp, ending at maturity.

Pericarp enlargement is accounted for by ADDOMS, NIGHTINGALE, and BLAKE (1) as being associated with cell division during the first 30 days and subsequently with cell expansion.

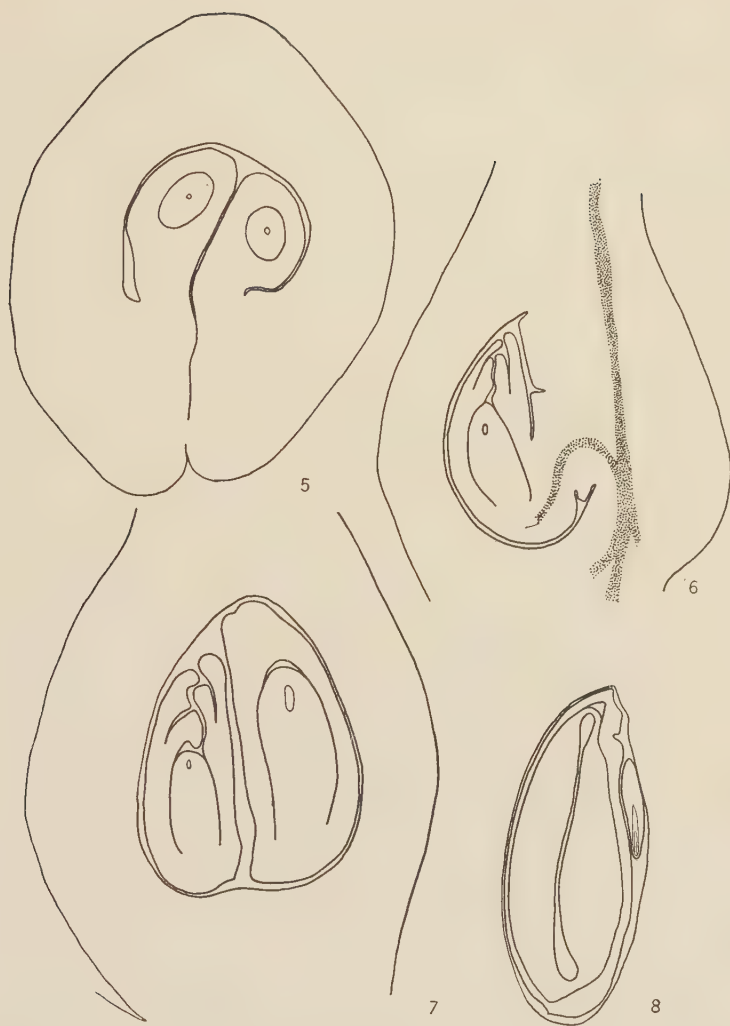
During the second period of growth (fig. 1), hardening of the inner wall of the pericarp takes place, forming the "pit."

NUCELLUS AND INTEGUMENTS

Two anatropous ovules are differentiated within a single carpel (figs. 5, 6). These two ovules develop equally until about three days before full bloom, when one of them gains the ascendancy and the other ceases to develop, so that at least a day before full bloom the latter may be recognized by its smaller size (fig. 7). The non-functional ovule is first recognized in stained sections by a peculiar transparency of the nucellus, while at the same time the gametophyte within appears normal. Degeneration involves shrinkage of the nucellus and a loss of characteristic organization by the gametophyte. The nucleoli of the latter apparently persist longer than do the other parts. These findings agree with those reported for the sour cherry (5).

The condition of a non-functional ovule at the time of full bloom is illustrated in figure 13. The latter is from the same carpel that contains the functional ovule shown in figure 25. Cessation of development of the non-functional ovule may occur at any point between the megaspore mother cell stage and the various stages in development of the gametophyte. Development of the functional ovule proceeds rapidly after full bloom, so that differences between the functional and the non-functional ovule become increasingly apparent. Figure 8 represents the relative development of the two ovules two weeks after fertilization.

The enlargement of the seed parallels the development of the pericarp during the first period of growth, so that by the close of the



FIGS. 5-8.*—Fig. 5, transverse section of pistil at time of full bloom; $\times 135$. Fig. 6, longitudinal section of pistil at time of full bloom; $\times 135$. Fig. 7, functional and non-functional ovules at time of full bloom; $\times 135$. Fig. 8, relative development of two ovules two weeks after fertilization, showing elongation of embryo sac; $\times 45$.

* All drawings were made with the aid of an Abbé camera lucida.

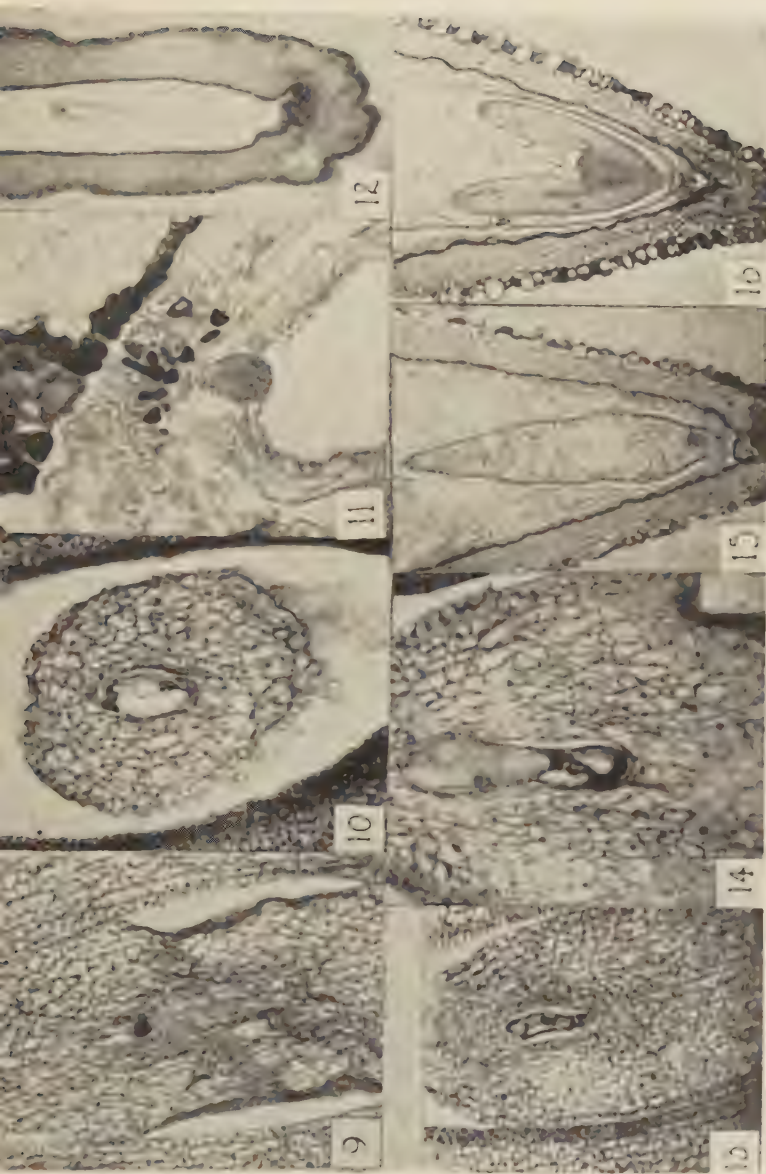
8-week period after bloom the integuments have attained a final and maximum size of about 20 mm. (fig. 1). The early maturation of the integuments has been recognized by many workers, including PÉCHOUTRE (11), TUKEY (12), DORSEY and McMUNN (8), and CONNORS (3). The maximum size of the integuments is reached about two weeks after cellular endosperm appears and is approximately coincident with an appreciable enlargement of the embryo.

The two integuments of the peach are distinct for only about one-fourth of their length (fig. 6).

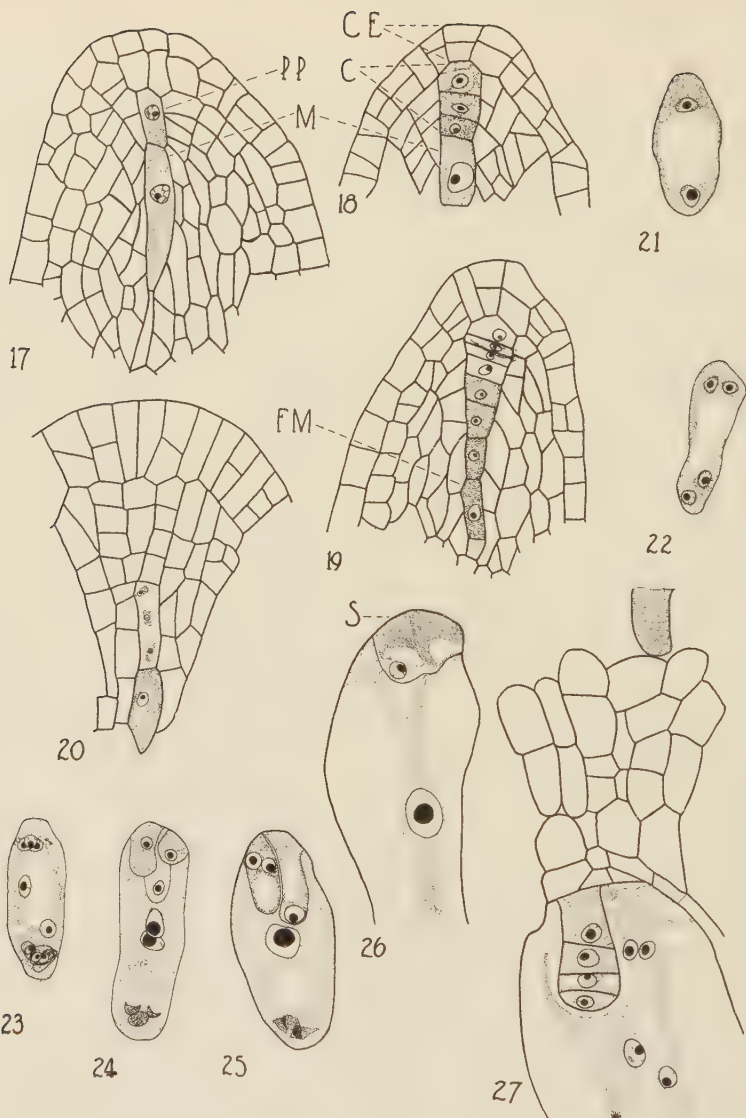
MEGAGAMETOPHYTE

The megaspore mother cell (fig. 18) is imbedded in the nucellus, being covered with three or four parietal cells and two or three layers of cells derived from the epidermis of the nucellus at the micropylar end (coiffé épidermique and calotte respectively of PÉCHOUTRE 11). Reduction divisions resulting in a linear tetrad of megaspores (fig. 19) occur a few days before bloom. The chalazal megaspore develops and the remaining megaspores rapidly disintegrate (fig. 20). The three divisions of the functional megaspore (figs. 21, 22) follow in rapid succession, so that a day or two before full bloom the megagametophyte (fig. 23) is in the 8-nucleate stage. Maturation and migration of the two polar nuclei to the middle of the megagametophyte (fig. 24) is practically coincident with full bloom. The two polar nuclei unite (fig. 25) during the period between full bloom and fertilization. The three antipodal nuclei are short-lived (fig. 25), being well along toward disintegration at the time of polar nuclear fusion. The synergids persist for about a week after fertilization.

The embryo sac begins to elongate following the union of the polar nuclei, doubles in length by the time of fertilization, and within two weeks extends almost to the chalaza (fig. 8). The embryo sac is characteristically swollen at the chalazal end, and owing to its narrow connecting canal forms a more or less dumb-bell shaped structure. TUKEY (12) reports the membrane surrounding the elongated sac to be continuous and persistent. According to BRADBURY (5) and DORSEY (7), when fertilization is prevented elongation of the embryo sac takes place, extending as much as two-thirds of the distance to the chalaza.



FIGS. 9-16.—Fig. 9, portion of ovule from aborting pistil of first drop, after full bloom; $\times 400$. Fig. 10, portion of ovule from aborting pistil of first drop, after full bloom; $\times 400$. Fig. 11, embryo and endosperm development in a developing fruit at time of second drop; $\times 400$. Fig. 12, embryo, endosperm, nucellus, and integument development in aborting fruit of second drop; $\times 60$. Fig. 13, portion of non-functional ovule at time of full bloom (same carpel as in Fig. 21); $\times 400$. Fig. 14, portion of ovule showing primary endosperm nucleus (cytote in adjoining section) of fruit of first drop, apparently "healthy." Note shrinkage of nucellus at chalazal end; $\times 400$. Fig. 15, portion of ovule showing early cellular endosperm, 4 weeks after full bloom; $\times 60$. Fig. 16, portion of ovule of developing fruit at time of third drop. Note embryo and cellular endosperm; $\times 60$.



FIGS. 17-27.—Fig. 17, portion of nucellus of ovule prior to full bloom, showing primary parietal cell (*pp*) and megaspore mother cell (*m*). Fig. 18, portion of nucellus of ovule prior to full bloom, showing coiffé épidermique (*ce*), three parietal cells (*c*), and megaspore mother cell (*m*). Fig. 19, portion of the nucellus showing four megaspores and functioning megaspore (*fm*). Fig. 20, portion of nucellus showing functioning megaspore and three disintegrating megaspores. Fig. 21, megagametophyte at 2-nucleate stage. Fig. 22, same at 4-nucleate stage. Fig. 23, same at 8-nucleate stage. Fig. 24, megagametophyte at time of full bloom. Note disintegration of antipodals. Fig. 25, megagametophyte just prior to fertilization. Note fusion of polar nuclei and their proximity to megagamete. Fig. 26, embryo sac following fertilization. Note disintegrating synergids, zygote, and primary endosperm nucleus. Fig. 27, portion of nucellus two weeks after fertilization. Note remains of pollen tube, 4-celled embryo, and numerous free nuclei of endosperm. All $\times 1860$.

FERTILIZATION

Double fertilization as described by BRADBURY (5) and reported by TUKEY (12) is found also in the peach. Evidence of fertilization was found in material in which the style had begun to fade, which was collected four days after full bloom. Prior to fertilization, the megagamete and the fused polar nucleus take positions close together (fig. 25). For a short period after fertilization the one-nucleate embryo and one-nucleate endosperm remain close together; then begins a gradual movement of the primary endosperm nucleus down the embryo sac toward the chalaza.

ENDOSPERM

Following fertilization, the endosperm remains uninucleate for a period of nine or ten days (fig. 26). The first division of the primary endosperm nucleus takes place at a point nearly one-half of the distance to the chalaza. Following the first division there occur in rapid succession numerous divisions of endosperm nuclei, so that the embryo sac is soon lined with a peripheral layer of free-nuclear endosperm (fig. 27).

This coenocytic condition persists until about the end of the sixth week after bloom. Walls begin to appear at that time at the micropylar end of the embryo sac. Development of cellular endosperm is rapid, so that during the following five weeks it comes to extend to the chalaza. This development takes place during the period of retarded pericarp enlargement (fig. 1).

EMBRYO

The zygote nucleus does not divide until about 12 days after fertilization, at which time there have been many divisions of the endosperm nuclei. Embryonal development is extremely slow until about a week before the beginning of the period of retarded pericarp growth and until after considerable cellular endosperm has been formed (fig. 15). The early retardation of the embryo has been noted by CONNORS (3), and by DORSEY and McMUNN (8); and by TUKEY (12) in the sweet cherry. Acceleration of the embryonic growth begins about the time that the cellular endosperm has reached one-third to

one-half the length of the nucellus. Cotyledons (fig. 16) are differentiated and the embryo quickly extends almost to the chalaza, digesting cellular endosperm and nucellus before it, and completing its elongation approximately 12 weeks after bloom (fig. 1). Following the attainment of full size of the embryo, the final 3-week period of pericarpic enlargement occurs.

Microscopic study of aborting fruits

FIRST DROP

The buds which fall before, during, and after full bloom have pistils in various stages of development and degeneration, which have apparently proceeded normally up to a point between the megaspore mother cell and the early gametophyte stages. Growth then stops and degeneration begins. Buds which fall in the first drop contain pistils in which both ovules have ceased to develop. The degeneration of the second ovule closely resembles that of the first. This check in development of both ovules before opening of the flower is recognized in the plum by DORSEY (7) and in the sour cherry by BRADBURY (5).

Flowers which fall with expanded petals contain ovules which have proceeded still further in development, usually being found in the two-, four-, or eight-nucleate stages of the megagametophyte. These megagametophytes evidence nuclear and cytoplasmic degeneration such as has been described for the non-functional ovule.

Flowers without petals also fall in the first drop. As a rule ovules of these pistils indicate that development has proceeded to a still further degree than that which has been described for those with petals. In this group are found ovules in which degeneration apparently began at the mature megagametophyte stage (fig. 10). Other ovules in this group give evidence of fertilization having taken place (fig. 14). In contrast to this, DORSEY (7) accounts for the entire drop on the basis of pistil abortion. The conditions shown in figures 10 and 14 are described by BRADBURY as being found in the second drop, in cases in which an embryo is not present. In certain cases (fig. 14) there is evidence of a shrinking of the nucellus at the chalazal end, while the embryo sac is apparently normal.

SECOND DROP

Fruits which fall in the second drop are among those which were considered as developing during the course of the first drop. Growth has proceeded to a further degree, the general relations of which are shown in figure 1. This drop occurs during the time of free-nuclear endosperm and early embryonal stages of developing fruits. The condition of embryo and adjacent endosperm is shown in figure 11, while the conditions of the corresponding drop is shown in figure 12. There is apparently no lack of development of integuments shown in the dropped fruits, the chief differences between the developing and aborting fruits being found in the nucellus, endosperm, and embryo.

DORSEY (7) reports this drop to be a result of lack of fertilization, while BRADBURY, on the contrary, finds that in some cases fruits of the second drop do have embryos and in other cases do not. Fruits in which pollination was prevented were found by both DORSEY and BRADBURY to fall in the first and second drops.

THIRD DROP

The embryos from fruits which fall in the third drop exhibit no apparent structural differences from those of developing fruits (fig. 16). DORSEY (7), BRADBURY (5), and TUKEY (12) report that fruits which fall in the third drop contain apparently normal embryos which, for some reason, have ceased development, size differences being the only observable ones between developing and aborting embryos. Fallen fruits contain seeds in which the integuments have begun to turn brown at the chalazal end, in contrast to no browning of those of seeds of developing fruits. This condition of browning is reported by DORSEY (7) as being evident both in the second and the third drops of the plum.

Discussion

The only apparent difference between aborting fruits and developing fruits is that growth has stopped in one and has continued in the other. Thus differences between developing and aborting fruits at the time of falling exhibit mainly degrees of development, and in some cases degeneration of normal parts. At the exact moment of cessation of development, there should be no structural differences

between fruits that are to develop to maturity and those that are to abort. In the first drop is found cessation of development at stages leading up to the gametophyte, also of gametophytes and of zygote and primary endosperm nucleus; and in the second and third drops a cessation of development of embryo and endosperm, which agrees in the main with the report of BRADBURY (5). This differs from the report of DORSEY (7) in that the first drop includes more than only "aborted pistils" and that the second drop results from more than "lack of fertilization," but agrees in regard to the third drop.

Granting the fact that development proceeds normally until the time of abortion, a diagram (fig. 1) may aid in the prediction of the approximate time at which development ceases, and the degree of development of the parts of each dropped fruit. Thus if the fruits of the second drop measure 8 to 14 mm. in length, it is reasonable to expect that growth stopped at about the time that most of the fruits had attained this measurement, which the chart shows to be approximately April 6-13. It is inferred also that these fruits fell approximately April 17-26. According to the diagram, the fruits which developed until April 6-13 possessed free-nuclear endosperm and a slight degree of embryonal development. The material shows this to be true. Likewise in the third drop the chart predicts cellular endosperm and additional embryonal development, which also is confirmed by the material.

TUKEY (13) has demonstrated that if the embryos from an early variety of sweet cherry, which does not produce viable seed, are placed under the proper conditions of artificial culture they continue to develop. As described by him, the embryos of the fruits which were checked in development before maturity of the embryo were in many ways similar in condition to the embryos contained in fruits of the third drop of the peach, the chief difference being that TUKEY (12, 13) was working with aborted embryos of developing fruits and not aborted embryos of dropping fruits. If it were possible to demonstrate that embryos of fruits falling in the third drop could be artificially cultured, then it would appear that the cause of the third drop was not due to the fact that the embryos ceased to develop, but rather as a result of some other factor.

DORSEY (7) observed in the second drop of the plum that the integuments begin to turn brown at the chalazal end, and that there is a shrinkage of the nucellus, before the pistil falls, and in the third drop that the nucellus is shriveled and "early browning of the seed coats soon follows suppression of the embryo." BRADBURY (5) found associated with cessation of development in each of the three drops a shrinking of the nucellus. TUKEY (12) inferred that inasmuch as in the early-ripening non-viable seed varieties of sweet cherry the embryos are often proportionately larger than the nucellus and integuments (a condition not found in viable seed varieties), perhaps "a check in the development of integuments and nucellus precedes a check in the development of the embryo."

Cases such as shown in figure 14 represent a situation in which apparently degeneration of the nucellus in the region of the chalaza precedes any change from normal in the embryo sac. These observations lead toward the idea that degeneration, and possibly cessation of development in fruits which will drop, begins at the chalazal end of the ovule. The seeds of fruits which fall in the second and third drops are brown at the chalazal end, the browning extending toward the micropylar end as degeneration proceeds. These observations suggest that in the course of abortion development stops first in the region of the chalaza and extends from there to the other parts of the seed and fruit. Such a disorder in the chalazal region would disarrange the vascular system, and might mean that development of the entire fruit stops because of a failure of conduction. This suggests that possibly the fruit falls, not because the embryo aborts but because these events are occasioned by an upset of the vascular system in the region of the chalaza.

Summary

1. The development of the megagametophyte and fruit of the peach agrees with facts established for other members of the genus *Prunus*.

2. The dropping of pistils occurs in three distinct waves during the first growth period, and each wave bears a constant relation to the developing pistils.

3. The aborting fruits differ from developing fruits mainly in degree of development of normal parts.

4. The possibility is indicated that a disorder in the region of the chalaza may occur prior to cessation of development of gametophyte or of embryo, resulting in subsequent degeneration of the seed and fruit.

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